of apamin-sensitive current between species may underlie different physiological roles.

3103-Pos Board B208

Plasticity in KCNQ1 Subcellular Distribution and Partnership with Different KCNE Subunits Contribute to Variations in IKs Channel Function in the Heart

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Background: KCNQ1 can associate with KCNE1 - KCNE5 subunits, singly or in combination, and assume different channel phenotypes in current amplitude and gating kinetics. In the heart, KCNQ1 is known to partner with KCNE1 to form the slow delayed rectifier (I_{Ks}) channel, a major contributor to cardiac electrical heterogeneity and stabilizer of heart rhythm. It is unclear whether KCNQ1 can partner with other KCNE subunits expressed in the heart, and how these potential partnerships impact on I_{Ks} channel function. **Methods:** We study the above issues by patch clamping, immunoblotting, and immunofluorescence/confocal microscopy. **Results**: (1) I_{Ks} current density is higher in guinea pig (GP) atrial (A) than ventricular (V) myocytes. There are 2 contributing factors: KCNQ1 protein level is higher in A than in V, and KCNQ1 is colocalized with KCNE1 in the peripheral cell surface to a higher degree in A than in V. (2) I_{Ks} current density in V myocytes is much lower in spontaneously hypertensive rat (SHR) than in GP, despite abundant expression of KCNQ1 and KCNE1 in SHR. This coincides with a higher degree of KCNQ1 colocalization with KCNE2 in SHR than in GP V myocytes, and the current-suppressing effect of KCNE2 on I_{Ks}. (3) I_{Ks} is *upregulated* during aging in SHR V myocytes. This correlates with a modest increase in KCNQ1 protein expression and, importantly, a prominent redistribution of KCNQ1 and a higher degree of colocalization with KCNE1 on the peripheral cell surface. **Conclusion**: We propose that the KCNQ1 channel exhibits plastic subcellular distribution patterns and dynamic partnerships with KCNE1 and KCNE2 in the heart. These factors contribute to I_{Ks} channel remodeling in response to variations in work load or changes in the heart rate.

3104-Pos Board B209

Sialytransferase ST3Gal4 Deficient Mice Demonstrate Left Ventricular Action Potential Extension and Attenuated IK

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Extracellular protein glycosylation is a ubiquitous cellular process that is vital to normal physiology. Aberrant glycosylation of both genetic and acquired etiologies are observed in severe disease states including congenital disorders of glycosylation and Chagas disease, which afflict millions. Cardiomyopathies including altered electrical signaling are a hallmark of these glycosylation disorders; although, little of the underlying mechanisms are understood. Cardiac conduction and contraction is dependent on various types of voltage-sensitive ion channels that are extensively modified by protein glycosylation. Our lab and others have demonstrated an isoform-specific role of glycosylation in channel gating. Protein glycosylation is a sequential process that involves hundreds of genes that are regulated throughout development and our lab showed that regulated glycosylation alters cardiac electrical signaling. One such glycogene, ST3 beta-galactoside alpha-2,3-sialyltransferase 4 (ST3Gal4), adds negatively charged sialic acids to many membrane proteins including voltage gated ion channels that likely contribute to the extracellular surface charge. Myocytes isolated from the left ventricular apex of ST3Gal4 deficient mice (n=5) demonstrated a 30 % ($p<0.05$) increase in the APD_{90} compared to gender and age matched controls (n=8). The current densities of both the peak and the slowly inactivating sustained (I_{Kslow}) repolarizing voltage-sensitive potassium currents were attenuated ($n<0.05$) at all depolarizing potentials in the ST3Gal4 mice (n=6) compared to controls (n=13). This reduction in potassium current should act to extend the action potential, as observed. The fact that the absence of a single gene involved in protein glycosylation can lead to such deleterious effects on cardiac electrical signaling may provide insight into other diseases of glycosylation that affect millions world-wide.

3105-Pos Board B210

A KCNE1 C-Terminus Long QT Mutation Disrupts a Crucial Interaction with the Kv7.1 Coiled-Coil Helix C and Reduces I_{KS} Channel Expression

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Kv7.1 α-subunit assembles with the KCNE1 auxiliary subunit to form the cardiac I_{KS} K^+ channel. Mutations in these subunit genes produce the long QT syndrome, a life-threatening ventricular arrhythmia. We recently explored the direct interactions between the C-termini of Kv7.1 and KCNE1 using purified recombinant proteins in a series of *in-vitro* pull-down experiments. We found that the KCNE1 C-terminus physically interacts with the coiled-coil helix-C of the tetramerization domain. Here we show that the missense LQT5 mutation (P127T) located in the KCNE1 distal C-terminus and previously found to produce a dominant Romano-Ward LQT syndrome, disrupted the physical interaction of KCNE1 with the Kv7.1 coiled-coil helix C. When co-expressed in CHO cells with WT Kv7.1, the KCNE1 LQT mutant P127T dramatically reduced I_{KS} current density, without altering channel gating properties. Our results show that deletion of the proximal KCNE1 C-terminus (KCNE1Δ69-77) significantly enhanced the binding of KCNE1 to Kv7.1 C-terminus. In contrast, deletions of the more distal regions of KCNE1 C-terminus (KCNE1Δ78-129 and KCNE1Δ109-129) totally prevented its binding to the Kv7.1 C-terminal region. Our data suggest that the most distal region of the KCNE1 C-terminus (aa.109-129) is crucial for the KCNE1 interaction with the Kv7.1 coiled-coil helix-C and that the missense LQT5 mutation P127T located within this domain affects I_{KS} channel expression by disrupting this coiled-coil interaction and possibly disturbing channel trafficking.

3106-Pos Board B211

Evolution of KChIP2 Gene Function is Localized Within the Core Promoter and 5' UTR

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The primary means by which different electrophysiological phenotypes are produced in different species is through the evolution of the cis-regulatory function of ion channel genes. The specific changes underlying this evolutionary process have proven elusive. We have begun to understand this process through analysis of the KChIP2 gene.

The KChIP2 gene encodes an auxiliary subunit obligatory for the expression of the transient outward current (I_{to}) in heart. KChIP2 mRNA expression levels are strongly correlated with the level of I_{to} expression in ventricular myocytes. I_{to} and KChIP2 mRNA expression is high in small rodents, such as mouse and rat, intermediate in larger mammals, such as human and canine, and largely absent in a few species, including guinea pig.

Gene function was studied using three species: mouse, human and guinea pig, representative of high, low and close to zero KChIP2 mRNA expression levels in heart, respectively. The KChIP2 promoter is a CpG island promoter and the transcription start site (TSS) maps to the same homologous region in all three species. The function of equivalent regions of the KChIP2 proximal promoter from mouse, guinea pig and human genomic sequences were compared using an in vitro transcription assay in cultured myocytes. There was a remarkably good correspondence between the transcriptional activity of this region of DNA and the relative level of mRNA expression in vivo. A series of deletion and swapping experiments demonstrated that the large differences in promoter activity were primarily due to sequence differences located within the core promoter and 5' UTR of the gene.

Surprisingly, evolution of core promoter function, rather than cardiac specific enhancer elements, primarily accounts for the large differences in KChIP2 expression in the ventricle of different mammalian species.

3107-Pos Board B212

Temperature Dependence of Herg Blocker Pharmacology - an Automated Patch Clamp Study

Cristian Ionescu-Zanetti, **Qin Chen**.

The hERG ion channel (Kv11.1) is at the center of preclinical cardiac safety assessment. Historically, patch clamp measurements that are used to assess hERG block of candidate compounds have been done at room temperature. While recording at physiological temperatures is challenging in terms of establishing and maintaining a high sealing resistance, recordings at elevated temperature are feasible using recently introduced automated patch clamp systems are capable of controlling temperature.

In this study we compare hERG current voltage relations and kinetics at different temperatures, and report observed changes in the pharmacology of known blockers using a next generation automated patch clamp platform.